

Flow-correlated dilution of a regular network leads to a percolating network during tumor induced angiogenesis

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We study a simplified stochastic model for the vascularization of a growing tumor, incorporating the formation of new blood vessels at the tumor periphery as well as their regression in the tumor center. The resulting morphology of the tumor vasculature differs drastically from the original one. We demonstrate that the probabilistic vessel collapse has to be correlated with the blood shear force in order to yield percolating network structures. The resulting tumor vasculature displays fractal properties. Fractal dimension, microvascular density (MVD), blood flow and shear force has been computed for a wide range of parameters.

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I. INTRODUCTION

A tumor growing beyond a critical size can only do so by remodeling its surrounding blood vessel network that supply nutrients and oxygen and removes waste products. This process is initiated by tumor cells secreting various growth factors that induce angiogenesis, the formation of new blood vessels from existing ones [1, 2, 3, 4]. The neo-vascularization primarily occurs in the periphery of the tumor which gives rise to a peripheral region with substantially increased Micro-Vascular Density (MVD) [5, 6]. Inside the tumor the MVD is usually drastically decreased, often leading to a necrotic core.

Previous studies by Gödde *et al* [7] focused on the angiogenesis showed that blood shear stress-dependent rather than pressure-dependent growth leads to a homogeneous distribution of capillaries. However, the influence of a growing tumor on the angiogenic vasculature was missing. Several other studies have focused on the angiogenesis in presence of a static tumor [8, 9] or growth of a tumor within fixed vasculature [10]. More recently biologically motivated hybrid cellular automaton models have been introduced by Bartha *et al*, Lee *et al* and Welter *et al* [11, 12, 13, 14] to study this remodeling process of the tumor vasculature via cooption, vessel growth and regression. Here the vasculature is modeled by a network of pipes carrying a flow that is a source for a oxygen or nutrient concentration field, whereas the tumor is modeled as a growth-and-death process that involves discrete cells proliferating or dying in dependence of the local level of this concentration field. Simultaneously the tumor cells are the sources for a growth factor concentration field that triggers the formation of new links in the vessel network at the tumor periphery and the dilation of pipes in the tumor's interior. Vessels can also regress inside the tumor due to increased solid stress or high acidity. Their models reproduce many known features of experimentally analyzed tumor vasculature like the compartmentalization into a highly vascularized perimeter, a well perfused tumor periphery and necrotic core with only a few thick vessels threading it [1, 6].

In addition to the inhomogeneous MVD, tumor vasculatures are abnormal in various ways, e.g., leaky, tortuous and dilated, have excessive branching and undergo constant regression and remodeling [4]. In particular it turned out that the emerging vasculature has fractal properties reminiscent of percolation clusters [15, 16, 17, 18]. In [11, 12, 13] it was argued that these specific geometric properties were the consequences of a flow correlated percolation process.

In order to disentangle the basic mechanism responsible for this process from the various biological details of the model described in [11, 12, 13] we formulate here a drastically simplified version of it, comprising, as we propose, the essential features leading to the global characteristics of the vessel network morphology. In essence we retain only the underlying dynamical pipe network capable of carrying a flow plus a circular tumor that increases its radius linearly in time. This circular region is further subdivided into a peripheral annulus where new pipes are inserted into the vessel network, and an inner annulus, where pipes are dilated and/or removed from the network. The latter process turn out to produce the flow correlated percolation process observed in [11, 12, 13].

The organization of the paper is as follows: In section II the model is defined, which represents a stochastic process that is studied with Monte Carlo simulation. The results of these simulations are presented and analyzed in the third section. In particular we study two variants of the model and demonstrate that only shear force correlated vessel collapse inside the tumor yields percolating morphologies of the tumor vasculature that is in good agreement with the experimental observations. Further, a fractal analysis of the resulting tumor vasculature is performed to quantify the network architecture. Section IV summarizes our findings.

II. DEFINITION OF THE MODEL

The model introduced in [11] and refined in [12, 13] consists of a network of pipes representing the vasculature and a cellular automaton, reminiscent of the Eden

TABLE I: List of variables

Variables	Description
L	System size
$l(e)$	Vessel length
t_c	Critical life time of vessel
R_T	Tumor radius
p	Vessel collapse probability
$P(e)$	Blood pressure at a given point
$Q(e)$	Blood flow per unit time
$F(e)$	Shear force on the vessel wall
MVD	Micro-Vascular Density

TABLE II: List of parameters (l.u.=lattice unit, a.u.=arbitrary unit)

Parameters	Description	Values
a	Initial vessel length	8 (l.u.)
$d(e), d_{\max}$	Vessel radius, Maximum value	1, 3.5(a.u.)
R_T^0	Initial tumor radius	30 (l.u.)
Δ_{angio}	Width of angiogenesis	15 (l.u.)
$p1, p2$	Angiogenic probabilities	0.01-0.5, p1/10
F_c	Critical shear for vessel collapse	0.5-0.7

model, representing the tumor. Both parts interact via a oxygen and/or nutrient concentration field, whose sources are perfused blood vessels, and a growth factor concentration field, whose sources are tumor cells. Both concentration fields are governed by diffusion equations. Blood flow through the blood vessels is modeled as ideal pipe flow with flow conservation in each node (junction) of the network. New vessels are inserted into the network under certain conditions, among which local growth factor concentration is the most important one. Vessels can vanish (regress) once they are overgrown by tumor cells. The latter proliferate when the oxygen/nutrient concentration is sufficiently large and die when it is too low. This model displays, over a wide range of the parameters, a growing tumor whose radius increases linearly with time. It displays a compartmentalization into a highly vascularized perimeter, a periphery region with increased vessel density and a necrotic core, i.e. a center in which most of the tumor cells are dead and which is threaded only by a few thick vessels surrounded by cuffs of tumor cells.

In the present study, we simplify the model drastically by discarding the oxygen and growth factor concentration fields. Unlike in [11, 12], where the tumor was represented by an eden cluster, we consider a circular tumor and linearly increasing radius with time. A highly vascularized region is observed in the proximity of the tumor's periphery [11, 12] which arises due to the interplay between the tumor secreted growth factor and existing vessel network. Inside the tumor core, vessels die as a result of complicated interactions with the tumor secreted growth factors and hypoxia [19, 20]. Our simplistic model, without considering such microscopic

details, mimics the essence of prior models [11, 12] by compartmentalizing the entire tumor into an annulus at the tumor periphery where new vessels can emerge and an inner annulus, where vessels can vanish with specialized criteria as discussed below. Also the processes by which new vessels are inserted is simplified to the extent of stochastic vessel growth. The model is defined as follows:

The model variables and model parameters are summarized in Tables I and II, respectively. The system configuration (tumor and network) is defined on a 2-dimensional graph $G = (V, E)$, where $V = \{v\}$ denotes the set of nodes and $E = \{e\}$ refers to the set of edges. Edge e , composed of ECs, describe vessels and node is marked by the junction where two or more vessels intersect. For simplicity we take the edges to be parallel to the coordinate axes. If $\vec{r}_1(x_1, y_1)$ and $\vec{r}_2(x_2, y_2)$ be the position vectors of the two end points along a vessel, then its length (edge length) is given by, $l(e) := |\vec{r}_1(x_1, y_1) - \vec{r}_2(x_2, y_2)|$, with $x_1 = x_2$ or $y_1 = y_2$. The graph G is then mapped onto an $L \times L$ ($= N$ sites) square lattice. The lattice spacing a denotes the initial vessel length which corresponds to $(L/a)^2$ unit square plaquettes (unit boxes) in the entire lattice.

Since our main focus is on the vasculature in the neighborhood of a growing tumor, and not on the structure of the tumor itself, the latter is only virtually present in the system. But its effective interaction with the vasculature is taken into account. The tumor T is essentially represented by the circular region of radius R_T centered at the lattice center.

In our model, blood vessel segments are represented by the edges. Each vessel is compared with a cylindrical rod of uniform circular cross-section of radius $d(e)$. The blood flow through the vessel is approximated by the laminar steady Poiseuille flow of a homogeneous, incompressible fluid (Newtonian fluid). Hence the amount of fluid $Q(e)$ that can pass through the vessel of length $l(e)$ per unit time is given by,

$$Q(e) = \frac{\pi}{8\eta} \frac{d^4(e)\Delta P(e)}{l(e)} \propto \frac{d^4(e)\Delta P(e)}{l(e)}, \quad (1)$$

where $\Delta P(e)$ is the pressure difference between the two ends of the vessel and η is the coefficient of dynamic fluid viscosity. Since η is assumed to be a constant, we renormalize the flow by the factor $\pi/8\eta$. The normalized shear force $F(e)$ acting upon the vessel wall, is given by,

$$F(e) = \frac{d(e)\Delta P(e)}{l(e)}. \quad (2)$$

The boundary condition for the pressure is chosen to establish a homogeneous flow and shear force distribution through each vessel-segments of the original network. We assume, the top-left corner ($x = 1, y = 1$) of the lattice at pressure P_0 ($=1$) is connected to the source artery, and the bottom-right corner ($x = L, y = L$)

at pressure 0 is connected to the sink vein. The pressure at any point on the boundary (e.g., top and bottom boundaries, $x = 1, \dots, L$ for $y = 1, L$; and left and right boundaries, $y = 1, \dots, L$ for $x = 1, L$ is given by: $P(x, y) = P_0[1 - (x + y)/(2L)]$. Using Kirchhoff's Current Law, given by the conservation of total current (compared with the blood flow in the present case) at a network junction, the blood pressure at every node is calculated. Substituting them in Eqs. 1-2 one immediately solves for the flow and shear force at every point of the network. The boundary condition we have chosen, ensures a global net flow in the diagonal ((1,1) to (L, L)) direction [11]. In the following, we will denote the flow and the shear force of the normal (undistorted) vessel network by Q_0 and F_0 respectively.

A. Initial configuration

The initial configuration of the system is described by uniformly spaced vessels of unit radius ($d_0(e) = 1$) in a square lattice [11] and a circular tumor-zone in the center. Initial Micro Vascular Density (MVD) is measured in terms of inter vessel distance a : $MVD = MVD_0 = \{e | \vec{r}(x_0 - x, y_0 - y), x, y \in (-a/2, a/2)\}$. Numerically, this is computed by estimating the total vessel length $l(e)$ (made by endothelial cells e), within an open box of width a . In our simulation we consider initial vessel length $l(e) = a = 8$ and hence $MVD_0 = 16$, which is half of the perimeter of a unit square plaquette of side a . Since, initially $d(e) = 1$ for the entire vasculature, as per Eqs. 1-2, both Q_0 and F_0 take values $\Delta P(e)/l(e) = 0.5/L$. The circular tumor, centered at $(L/2, L/2)$, has the initial radius $R_T^0 = L/20$. With these initial configurations, we now proceed to describe the following deterministic and stochastic rules which update the tumor and vasculature in each time step.

B. Tumor Growth

At each time step, the radius of the tumor R_T is increased by one lattice site, i.e., all sites outside the surface of the tumor with $\vec{r} = \{\vec{r} | \vec{r} \notin EC, TC; \vec{r}' \in TC; |\vec{r} - \vec{r}'| = R_T(t+1) - R_T(t)\}$ are occupied by tumor cells at time $t+1$. Although, the proliferation of TC must be supported by availability of adequate oxygen in the neighborhood of the tumor surface [11, 12], in our simplified model, such restrictions are not imposed. We assume that there is always sufficient oxygen available in the neighborhood and along the tumor perimeter region to assist the proliferation process. Tumor continues to evolve until the finite size of the simulating system restrict its growth at $R_T = L/2 - \Delta_{\text{angio}}$, where Δ_{angio} is the width of angiogenesis outside the tumor surface, as illustrated below.

C. Angiogenesis

Each tumor cell releases some growth factor (GF) which activates the proliferation of new blood vessels [1]. GF concentration is high inside the tumor and decays to a negligible concentration beyond a distance $R_T + \Delta_{\text{angio}}$. Naturally, Δ_{angio} is the range of angiogenesis. Since, the space inside the tumor is densely occupied by tumor cells, the vessel proliferation in that region is highly suppressed and therefore we consider the angiogenesis to occur mainly within the arbitrarily chosen annular ring of thickness $\Delta_{\text{angio}} = R_T^0/2$, outside the tumor surface. (We have repeated our simulation by considering the vessel proliferation within the annular ring or thickness $R_T - \Delta_{\text{angio}}/2$ to $R_T + \Delta_{\text{angio}}/2$ and found that the choice of Δ_{angio} does not affect the tumor morphology in large time limit: see D). New vessels are added into the system in the form of a “+” shaped plaquette. A random unit cell (box of edge length a) is chosen within the angiogenic regime, such that it is not occupied by an endothelial cell ($e = 0$) and then a “+” plaquette (type I) is embedded into unit the cell with probability p_1 (see Fig. 1). The insertion of type I plaquette splits a unit cell into four equal sub-cells of edge length $a/2$. At each time step this process is repeated $\pi[R_{\text{angio}}^2 - R_T^2]/a^2$ times which is equal to the total number of places where angiogenesis can possibly occur. Once a type I plaquette is embedded into a unit cell, it is divided into four equal sub-cells each with edge length $a/2$. The substructure is made more microscopic by inserting a second kind of plaquette, type II, of edge length $a/4$ with probability p_2 . Note that, a type II plaquette can be placed into the system, only when a type I plaquettes is available in the system. In this paper we present results for $p_1 = 0.05$ and $p_2 = 0.005$, unless otherwise specified. For these parameter values the resulting pattern of the vasculature appears to be similar to the one observed in physiological circumstances. A comparative study for different angiogenic probabilities have been discussed in section III C. In this study, we do not consider dynamic variations in angiogenesis during the course of tumor evolution, i.e. both p_1 and p_2 are constant in time. This approximation is reasonable, but might not be the case in real systems and should be addressed in our future models.

D. Vessel Dilation and Collapse

Inside the tumor angiogenic sprouting is minimized, however, endothelial cell proliferation still occurs along the vessel perimeter, leading to their enhanced circumferential growth. Experiments proposed that high growth factor and hypoxia induced activation of Eph/ephrin pathways [19, 20] might be responsible for vessel thickening inside tumor core. Thus in the present model, all vessels inside the tumor are updated sequentially by increasing their radius $d(e)$ at each time step by δ_d with a probability p_d as long as $d(e)$ does not saturates to

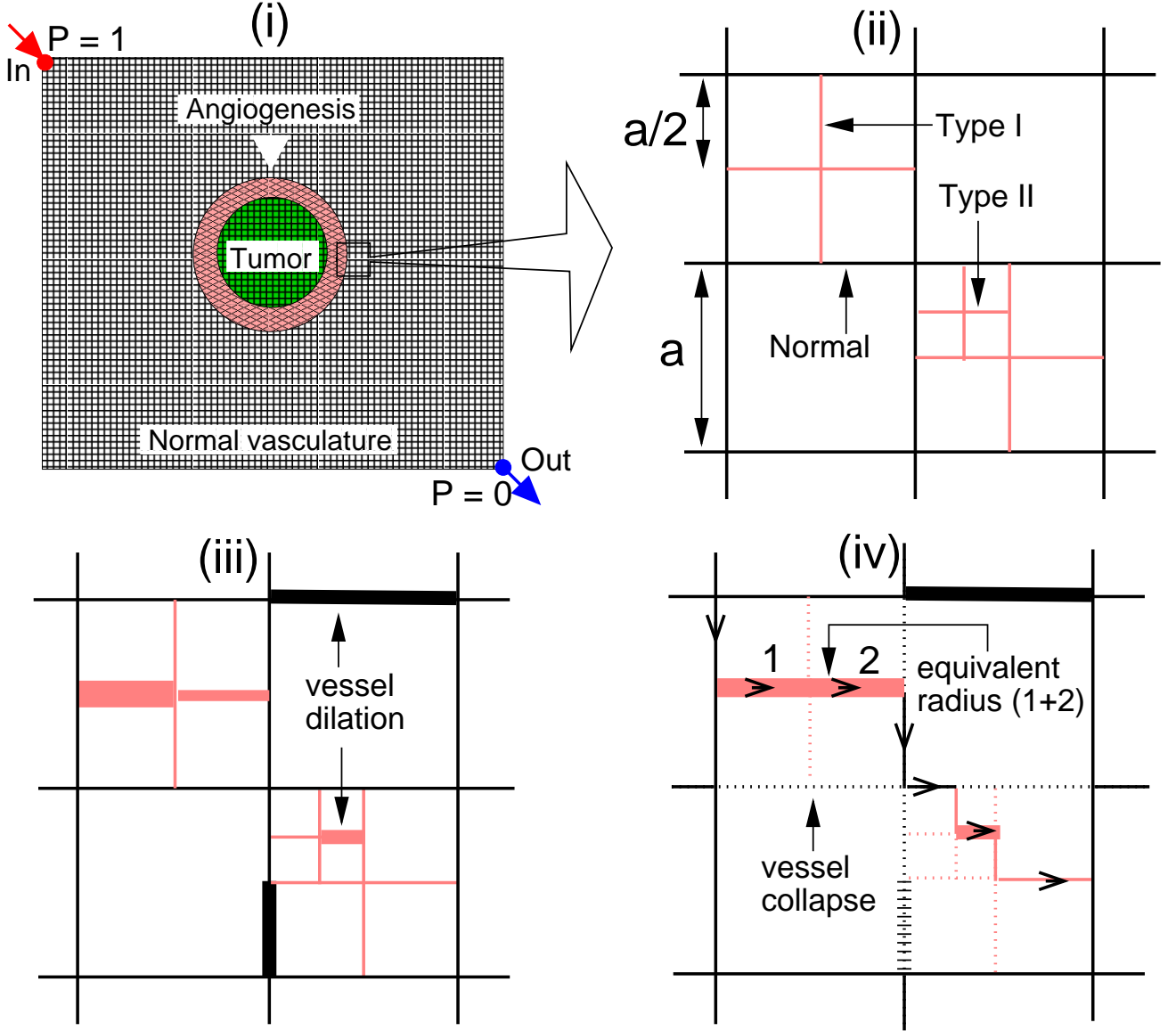


FIG. 1: (Color online) (i) Schematics of a tumor, angiogenic and normal vasculature. In our model the blood flow, if any, enters the system at the top left corner and exit from the bottom right corner. (ii) Magnified versions of the normal vessels and angiogenesis by inserting “+” shaped plaquettes within the unit (edge length a) and subunit cells (edge length $a/2$). (iii) Vessel dilation, shown by thick lines. (iv) Vessel collapse (dotted lines), i.e., disappearance of vessel existing in (ii) and (iii). Vessels marked with 1 and 2 of different radii may be replaced by a single vessel with an equivalent uniform radius. After a number of vessel collapse, a single, long, non-uniform vessel might appear (shown by successive arrows).

a maximum value d_{\max} : $d(e) := d(e) + \delta_d \Theta(\chi - p_d)$, if $d(e) < d_{\max}$, where χ is a random number and $\Theta(x) = 1$ for positive x and 0 elsewhere.

Tumor vessels are tortuous and leaky. Vessel membranes become unstable due the lack of pericytes, resulting in frequent vessel collapse and regression [11]. A reduced flow may not be sufficient to prevent the vessel against the stress exerted by the neighboring tumor cells and can become the key factor for their collapse [7]. We calculate the vessel shear force and identify weakly perfusing vessels inside the tumor: $e \in E = \{|\vec{r} - \vec{r}'| < R_T\}$. Vessels with normalized shear forces $F(e)/F_0$ falling be-

low the critical value F_c , are removed from the rest of the vasculature with probability p [11, 12]. Instead of removing the vessels by shear force criterion, if we remove them with normalized flow $Q(e)/Q_0 < Q_c$ (not shown), or normalized pressure (see Appendix C) a completely different vasculature is observed. Other than the flow, pressure and shear force correlated vessel collapse we have also employed another criterion for vessel collapse, in which the vessels inside the tumor, below a certain age (t_c =critical lifetime), are removed probabilistically. This mechanism suggests that, if vessels are able to survive until a critical life time t_c , they live for ever.

Both, vessel-dilation and vessel-collapse together give rise to long nonuniform vessels as shown in Fig. 1(iv). To make the calculations easier, non-uniform vessels with different radii $d_i(e)$ (see vessel 1 and 2 in Fig. 1 (iii)-(iv)) are assumed to be replaced by a single vessel with effective radius $d_{\text{eff}}(e) = [\sum_i 1/d_i^4]^{-1/4}$.

III. NUMERICAL RESULTS

The update rules described in the previous section, define a stochastic process which we study numerically. At time $t = 0$, we start with a system of regularized vascular network and a pre-existing circular tumor at the center. We have simulated our model over a wide range of parameters and applied various criteria for vessel collapse. In the following, however, we present results which produce realistic vessel morphologies reported in prior investigations [6].

A. Random vessel collapse inside the growing tumor leads to a “void” core

First we consider the growth of a circular tumor in the back-bone of a capillary network arranged in a $2d$ square lattice, as described earlier. The entire lattice is assumed to be connected to outer vessels (vein and arteries) along one of the two diagonal directions. During the tumor evolution, vessels inside the tumor collapse with a certain probability, resulting in many non-circulating dangling vessels. At each time step these non-circulating (non-biconnected) vessels are completely removed from the rest of the lattice. Vessels do not collapse further if they survive up to a critical lifetime t_c . It is easy to visualize that in the beginning, although all intra-tumoral vessels are in critical state, with the maturation (aging) of the tumor, critical zone moves away from the tumor center and stay limited within the annulus of thickness $(t - t_c)$ (for $t > t_c$) under the tumor surface. When the tumor diameter becomes comparable to the linear size L of the lattice, we examine if a continuous path exists inside the tumor along any of the two diagonal directions of the lattice. If such a path exists, the probability P_∞ of finding a spanning cluster is 1, and 0, otherwise. Slowly increasing the vessel collapse probability p from zero to a maximum value, we calculate P_∞ . A transition of the vasculature morphology from a dense to a void network is observed at the critical $p = p_c$. Two different cases have been studied in this context: vasculature I. *without* and II. *with* angiogenesis.

I. Vasculature without angiogenesis disappear at small collapse probability: Morphologies of the tumor vasculature for random vessel collapse and without angiogenesis are displayed in Fig. 2 (a), (b) and (c), for collapse probabilities below, above and at the percolation threshold respectively. We calculate P_∞ for different L , as a function of vessel collapse probability p . The re-

sults are plotted in Fig. 2 (d) for $t_c = 20, 30, 40$. There is no restriction imposed on the choice of t_c , since we have also observed percolation for various other values of t_c . At small t_c , quick vessel stabilization leads to a diverging percolation thresholds, whereas, at large t_c , rapid vessel collapse results in a converging percolation threshold. Thus, for the sake of clarity, we have not shown those data in Fig. 2. All our data, presented in Fig. 2 are averaged over 400 ensembles. A sharp decrease in the value of P_∞ characterizes the percolation transition at p_c , above which a spanning cluster ceases to exist in the system. For $t_c=20$, $p_c=0.004$, which decreases further as t_c is increased. The exponents $\nu = 4/3$ has been estimated from the finite size scaling analysis for all t_c (see inset of Fig. 2 (d) for $t_c = 20$) and its value is found to be independent of t_c .

II. Vasculature with angiogenesis remain percolating at higher collapse probability: Here we introduce angiogenic sprouting in the tumor surrounding vasculature by inserting type I and II plaquettes. The resulting tumor is found to be enclosed by a shell of high MVD, as depicted in Fig. 2 (e), (f) and (g). In a similar way, like in the previous case, we carry out measurement to find P_∞ for angiogenic probabilities $p_1 = 0.05$ and $p_2 = 0.005$ and plot the results in Fig. 2 (h). The value of $p_c \simeq 0.0056$ and $\nu = 4/3$ are estimated from the data collapse for $t_c = 20$, as shown in the inset of Fig. 2(h). It is readily observed that percolation transition for $t_c = 20$, in presence of angiogenesis, is shifted to a higher value at $p_c=0.0056$, from $p_c=0.004$, when there is no angiogenesis.

Our results corresponding to Fig. 2 suggest that random vessel collapse with and without angiogenesis yields two distinct phases of the tumor vasculature: one in which the tumor center is completely vascularized and one in which it is completely void, separated by a percolation transition at a critical collapse probability. Both phases appears to be unrealistic compared to the real tumors and prior research [11, 12], except the critical point itself when a few vessels threading the tumor center. This vasculature is similar to the reminiscent of a real tumor vasculature - but requires, in this model, a fine tuning of a certain parameter (the collapse probability), which is again unrealistic. In the following, we will present a mechanism by which the vessel network drives itself into such a critical state.

B. Shear force correlated vessel collapse inside the growing tumor leads to a “percolating” vasculature

We carried out a similar study of percolation, as described in the previous section, for the shear force correlated vessel collapse. Here we consider two different cases: tumor vasculature I) with uniform vessel and II) dilated vessel. In these cases, vessels neither undergo random collapse, nor stabilize automatically with their age (t_c), as considered in the previous section. (Note that, aging increases the stability of the vessels. To ver-

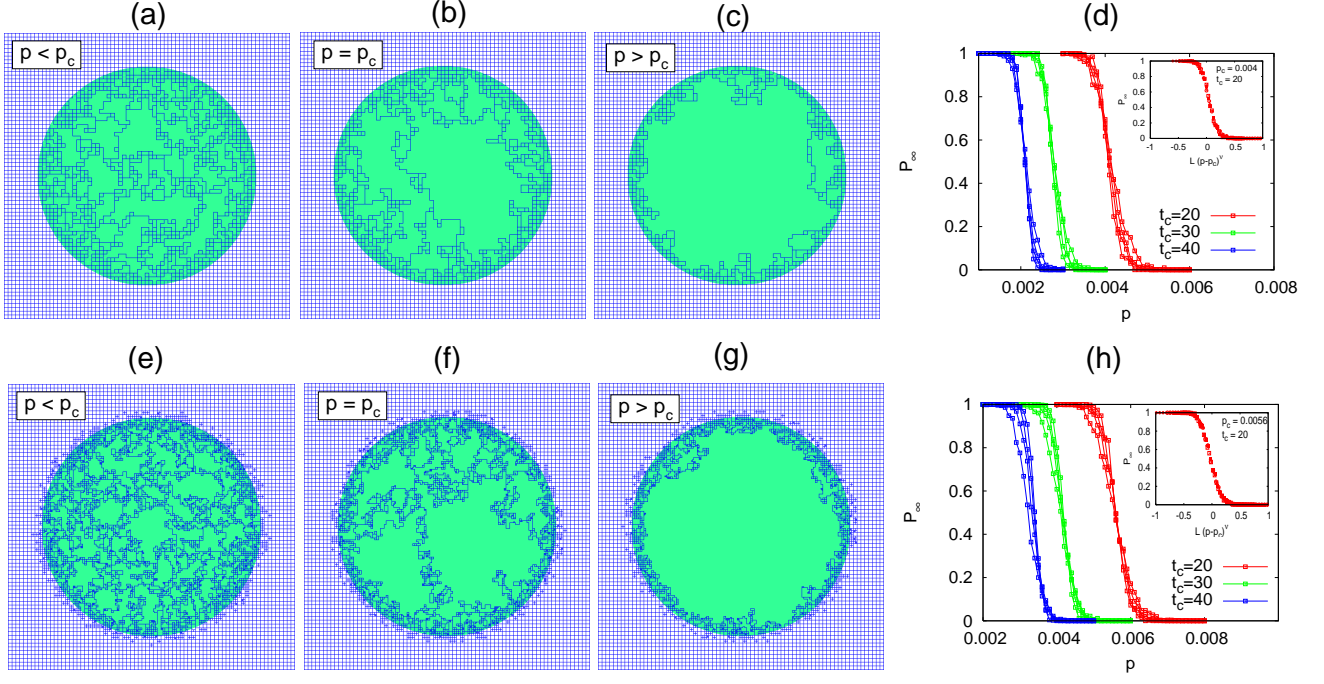


FIG. 2: (Color online) **Top row:** Snapshots of a growing tumor vasculature for random vessel collapse without angiogenesis. Circular green region represents the tumor and blue lines represent the blood vessels. Morphologies correspond to different collapse probabilities (a) below (b) at and (c) above the percolation threshold. In the subplot (d) P_∞ is shown as a function of collapse probability p for different values of critical vessel lifetime $t_c = 20, 30$ and 40 with systems sizes $L = 320, 400, 480$ and 600 . As t_c increases, vessel stabilization takes longer and the percolation threshold p_c goes down. **Inset (d):** Data collapse of the curves for $t_c = 20$ with $p_c \simeq 0.004$ and $\nu = 4/3$. **Bottom row:** (a) Snapshots of a growing tumor vasculature with angiogenesis. Probabilities for plaquettes are $p_1 = 0.05$ and $p_2 = 0.005$. Subplots (e), (f) and (g) represents vasculature below, at and above the percolation threshold respectively. In (h) P_∞ vs. p are shown for the same L and t_c , mentioned above. **Inset (h):** Scaled version of the data for $t_c = 20$ with $p_c \simeq 0.0056$ and $\nu = 4/3$.

ify the robustness of the vasculature in presence of aging, simulations are carried out and the results are shown in Appendix E.) Vessels are removed with normalized shear force falling below the critical value $F_c = 0.5$, and with collapse probabilities p varying from 0 to 1. The results are depicted in Fig. 3. In contrast with the previous results (void tumor at high p , as depicted in Fig. 2), our present analysis asserts that tumor interior is always percolating, no matter what the magnitude of the collapse probabilities are.

We now focus on the microstructures of the angiogenic vasculature evolving under shear correlated vessel collapse.

I. Lack of vessel dilation produces dense tumor vasculature: First we discuss the case when there is no vessel dilation occurring inside the tumor, i.e., vessel radius $d = 1$ is a constant. Vessels inside the tumor with shear force (as per Eq. 1, $Q(e) = F(e)$, since $d = 1$) falling under the critical value $F_c = F(e)/F_0 = 0.5$ are removed with probability $p = 0.025$. In Fig. 4 we show the snapshots of the evolving vessel network.

As time progresses, homogeneous vascular network with constant MVD throughout, is rapidly changed into an inhomogeneous one (see Appendix A). Angiogenesis in the peritumoral region produces a huge number of ves-

sel junctions, each of which divides the original flow into multiple components. The new vessels carry less blood resulting in a weaker shear force. Therefore they collapse as time goes by. Once the weak vessels are removed, remaining vessels carry more blood and stabilize against the collapse. In the late stage of the simulation, vessel strips exist along and transverse to the principal flow direction, accompanied by empty regions. However, under any circumstances, the final structure of the remaining vessels appear to be dense and bear little resemblance to the actual tumor vasculature.

II. Vessel dilation inside the tumor produces realistic tumor vasculature: So far, we have presented results for tumor vasculature with uniform vessel radius. The scenario becomes largely different once the vessels start thickening inside the tumor simultaneously with the angiogenesis occurring outside the tumor (see Appendix B). Starting with a homogeneous network of vessels, with uniform radius $d(e) = 1 \forall e$, probabilistic vessel dilation (with probability 0.025) for vessels with $d(e) < d_{\max} = 3.5$ are allowed to occur, inside the tumor. Intra tumor vessels, with shear force (as per Eq. 2) falling under critical value $F_c = F(e)/F_0 = 0.5$ are removed with collapse probability $p = 0.025$. Resulting vasculature morphologies are shown in Fig. 5 (for mea-

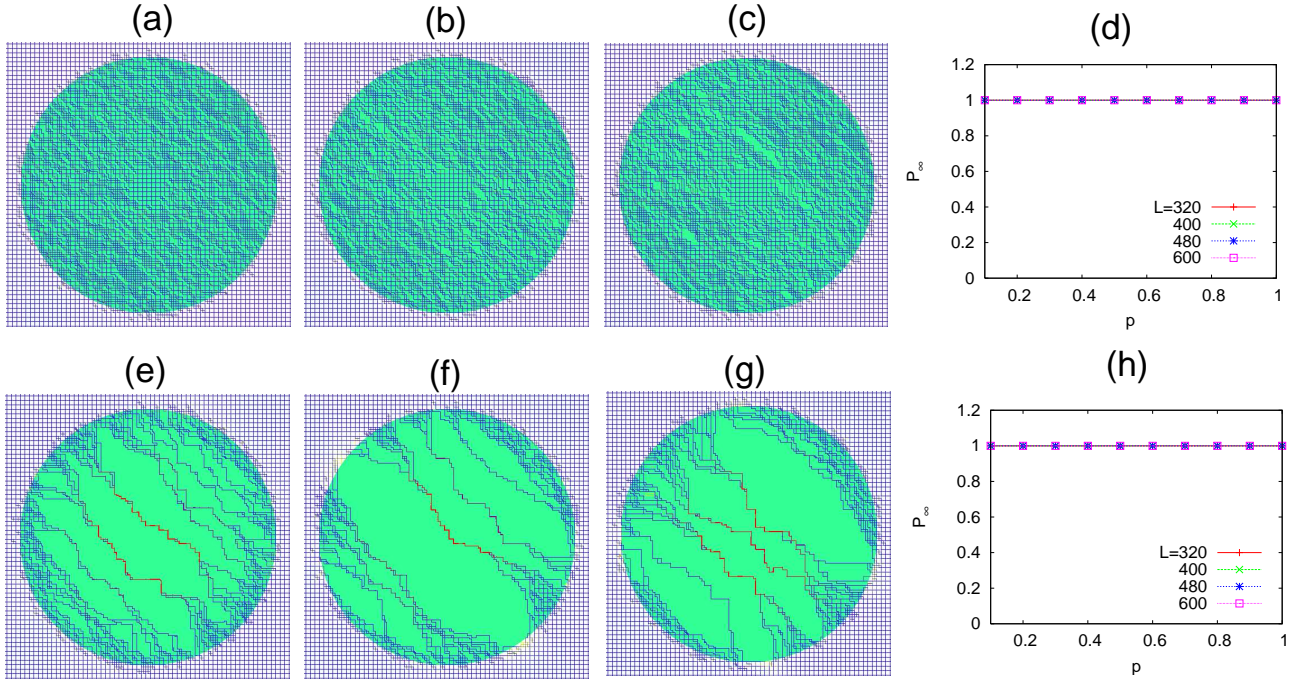


FIG. 3: (Color online) Snapshots of a growing tumor vasculature for shear force correlated vessel collapse. Tumor is shown in green. Blood vessels are colored according to their instantaneous blood flow: red indicates high (inside the tumor, along the flow direction: top-left to bottom-right), blue indicates normal (away from the tumor surface) and yellow indicates low blood flow. **Top row:** Uniform vessel inside tumor. Morphologies correspond to different collapse probabilities: (a) small (b) medium and (c) large. In the subplot (d) P_∞ is shown as a function of collapse probability p for $F_c = 0.5$ with system sizes $L = 320, 400, 480$ and 600 . **Bottom row:** Dilated vessel inside tumor, similar to the Top row. Vasculature is always percolating inside the tumor.

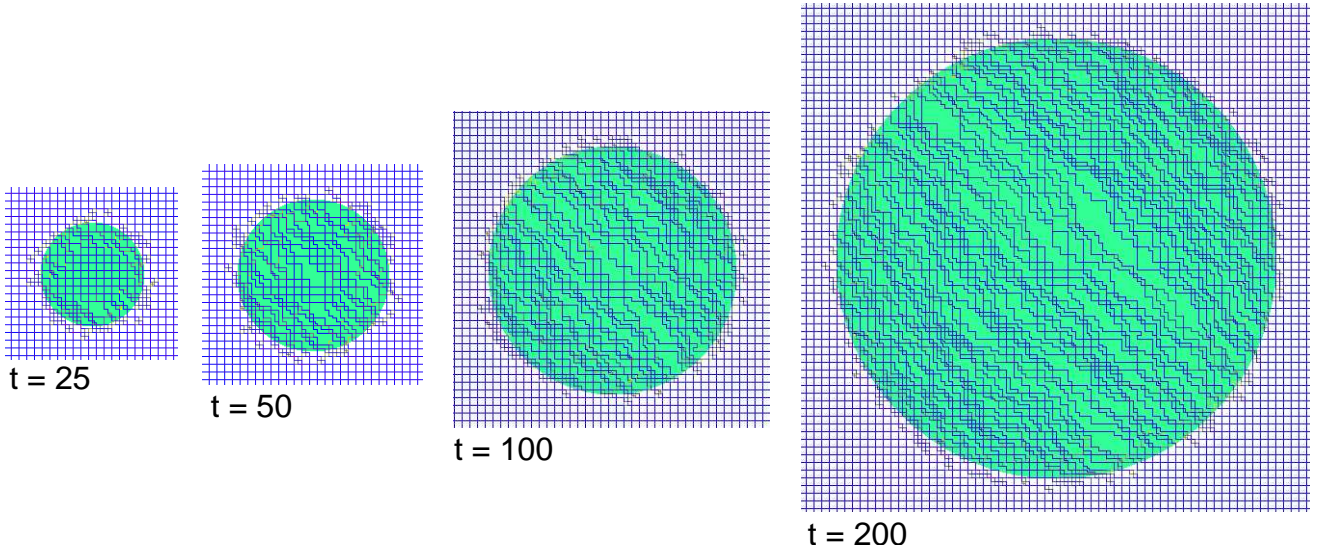


FIG. 4: (Color online) Snapshots of a growing tumor with uniform vessel radius and angiogenic probabilities $p_1 = 0.05$ and $p_2 = 0.005$ for linear system size $L = 600$. Vessels are collapsed under critical shear $F_c = 0.7$ with probability 0.025 . The color code is described in Fig. 3.

measurements MVD, shear force, vessel radius, pressure gradient, see Appendix B). One observes that, the interplay between angiogenesis and shear correlated vessel collapse rapidly changes the homogeneous vascular network into

an inhomogeneous one. Resultant vessels inside the tumor grow thick and become stable against any further collapse.

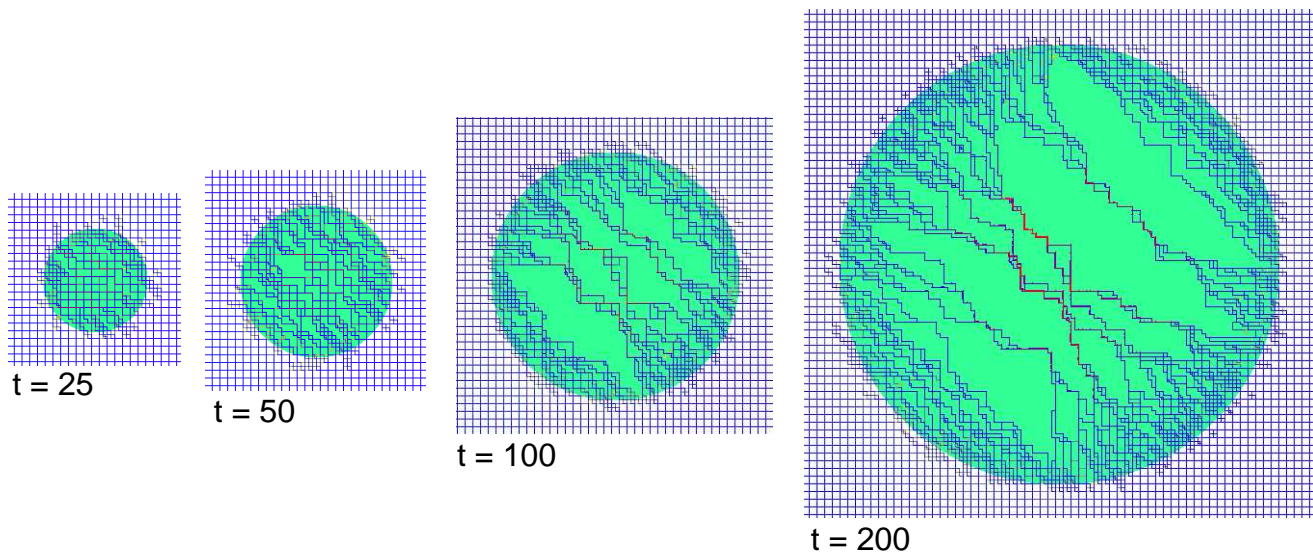


FIG. 5: (Color online) Snapshots of a growing tumor similar to Fig. 4, but with a probabilistic vessel dilation interior to the tumor. Vessels are collapsed under critical shear $F_c = 0.5$ with probability 0.025. The color code is the same as described in Fig. 3.

C. Flow correlated percolation influences the fractal vasculature

This section focuses on the geometrical aspects of the tumor vasculature. In the asymptotic time limit, one observes the turn over of a homogeneous vasculature into a completely inhomogeneous one, resulting from the interplay between angiogenesis and shear force determined vessel collapse. One remains with irregular vasculature with high MVD in the peritumoral neighborhood and a few long, thick vessels in the interior of the tumor, prevailing along the flow direction. It is still unclear, what are the main influencing factors that reshape the tumor vasculature. A detailed insight into the remodeling process of the vessel network would be given its fractal dimension. A fractal, in general, is a rough geometrical structure which is self similar or scale invariant. However, the emerging network in our study, displays a broken symmetry due to the diagonal global flow and thus spatially inequivalent regions arise. Estimation of the fractal dimension would shed light on the magnitude of spatial influence applied by the tumor on the vessel growth process. Therefore, it is worth to focus on the fractal properties and quantify the structural changes of the network in the tumor environment. The fractal dimension D_f of the vascular network is determined by the box counting method [22]. It is given by the ratio between the logarithm of the number of boxes needed to cover the vessel network (N_ξ) and the logarithm of the linear box size:

$$D_f = - \lim_{\xi \rightarrow 0} \frac{\ln N_\xi}{\ln \xi}. \quad (3)$$

We analyze the modified vasculature extending from the center of the tumor up to the angiogenic peritumoral re-

gions. Beyond this region the vasculature is normal. In our measurement, we divide our region of interest into several annuli with fixed outer radius, determined by the limit of the peritumoral plexus, and varying inner radius R_i . Fig. 6 displays the number of boxes N_ξ as a function of the box size ξ in a log-log scale. (Note that, the slope in Fig. 6 decreases as the box size ξ goes below the characteristic length scale of the system, set by the initial vessel length $a = 8$. Therefore, in the measurement of D_f those points are disregarded.) Fitting a straight line through ~ 11 data points (extending almost two decades), we find $D_f = 1.72 \pm 0.1$, corresponding to the entire vasculature remodeled by the tumor. Similar studies, as a function of the angiogenic probabilities result in little variation in D_f , although the morphology appears to be quite different (see Fig. 7). Our numerical estimate of D_f could be compared with the experimental prediction [15] obtained on a 2d slice of human carcinoma using 2d image analysis. Instead of studying the entire vasculature, if we consider annular rings of different thickness, near the peritumoral plexus we obtain $D_f = 1.50 \pm 0.02$. We observe only small changes of D_f , when the angiogenic probability p_1 is varied from 0.05 up to 0.5, and the range of angiogenesis Δ_{angio} is varied from a up to $3a$ (not shown).

Robustness of fractal vasculature under varying angiogenesis: Tumor vasculature is observed to be modified by the rate of angiogenesis. Therefore a quantitative study of the vessel network is carried out by varying the amplitude of angiogenesis. A similar study reported the variations of D_f as a function of critical shear force [13]. Our study, so far, addressed angiogenesis occurring with probabilities $p_1 = 0.05$ and $p_2 = p_1/10$. Keeping this ratio fixed, we now vary p_1 from 0.01 up to 0.5. The vasculature resulting at very late stage of the simulation are shown in Fig. 7(a)-(c). With increasing

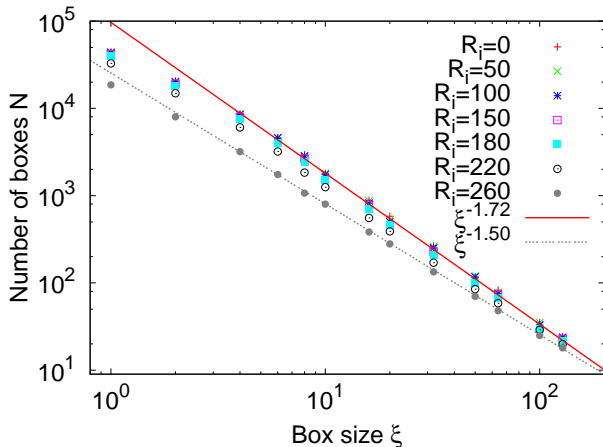


FIG. 6: (Color online) Box counting estimate of D_f of the tumor vasculature. We confined the measurement to annuli with fixed outer radius that is determined by the limit of the peritumoral plexus (extend up to the angiogenic region outside the tumor periphery) and with varying inner radius R_i . The number of boxes of size ξ needed to cover the vasculature within the annuli, is plotted in log-log scale. The slope of the curves correspond to D_f which decreases with increasing R_i : $D_f = 1.72 \pm 0.01$ for $R_i = 0$ (corresponds to the complete tumor and angiogenic vasculature) and $D_f = 1.50 \pm 0.02$ for $R_i = 260$ (corresponds to the angiogenic vasculature only), indicating that the fractal dimension is drastically modified inside the tumor.

angiogenesis, average blood shear force decreases in the interior of the tumor, giving rise to a fusiform, avascular region along the principal flow direction. However, vasculature through the tumor still remains percolating. We further analyze the fractal dimensions D_f of the obtained vessel networks, for which the results are plotted in Fig. 7(d). The fractal dimension for both the entire tumor vasculature ($R_i = 0$) and the annular angiogenic region ($R_i = 260$) remain almost constant in the regime of large $p_1 > 0.05$, however, decays for smaller p_1 . Our analysis confirms the robustness of the tumor vasculature under angiogenic perturbations.

IV. SUMMARY

Tumor induced angiogenesis is a basic mechanism in cancer development. We have presented a simple stochastic model which initiates with the regular vasculature that consists of capillaries of equal diameter arranged in a regular grid with a given MVD ensuring a homogeneous distribution of flow and constant shear stress in all constituting vessels. Once the tumor grows up to a certain size, the vasculature gets modified into characteristically different sections. Since the vessel segments need to be biconnected to the exterior network, a large part of the tumor vasculature can be cut from the rest of the network by only a few vessel collapse. The inhomogeneity

in the network extends from high MVD with thin vessel region in the peritumoral plexus up to a sparsely populated and thick vessel environment in the interior of the tumor [1, 6]. The spatio-temporal characteristic curves for MVD, blood flow, shear force, pressure gradient and vessel radius as predicted in [11, 12, 13], are reproducible by our simplistic approach (see Appendix B).

Our model predicts that inhomogeneity in the network is caused by the interplay between the excessive vascularization in the tumor periphery and vessel collapse in the interior. We have implemented two different mechanisms for the probabilistic rupture of vessels *viz.* random collapse and shear stress correlated collapse. The latter is motivated by the study on normal vessels which undergo a structural reduction of internal vessel diameter due to a decreased wall shear stress (brought about by changes of blood flow) [23]. When the vessels are removed randomly, the interior of the tumor is either containing a dense vasculature or completely empty. This phenomenon is well understood from the basic law of percolation theory [21] and suggest the existence of a spanning vasculature for a particular collapse probability $p = p_c$, known as percolation threshold. Under the random vessel collapse criterion, a percolating phase requires a fine tuning of p which depends strongly on the model parameters.

Next we considered a probabilistic vessel collapse, with shear force falling below a critical value inside the tumor, while the vessel diameter is kept constant. After the initial removal of weakly perfusing vessels, which are usually perpendicular to the flow direction, long vessels remain parallel to the principal flow direction. These vessels may further suffer from the insufficient shear force due to their increased length. However, an increased pressure drop across the vessel or an enlargement in vessel diameter can amplify the amount of blood flow and consequently the shear force. Vessels, for which the shear compensation is not made by the increased flow or pressure drop, eventually collapse. Thus, in the long run, a few empty regions appearing along the principal flow direction, with increased MVD regions visible along the transverse flow direction, producing a physically unrealistic tumor vasculature, unlike as reported in [1, 6, 11, 12, 13, 14].

Our model predicts a percolating morphology of tumor vasculature which is maintained by the correlation between the probabilistic vessel collapse and the local shear stress exerted by the blood flowing through it. However, they key ingredient to produce a realistic vasculature is still missing.

Therefore, we included the intratumoral vessel dilation. High growth factor and hypoxia induced activation of Eph/ephrin pathways has been reported to reduce the angiogenic branching and enhance the vascular circumferential growth inside the tumor [19, 20]. Vessel dilation which occurs inside the tumor [1, 6] and taken into account in prior models [11, 12, 13, 14], plays crucial role in determining the vasculature morphology. Implementing probabilistic vessels dilation inside the tumor together with shear stress correlated vessel collapse, a physically

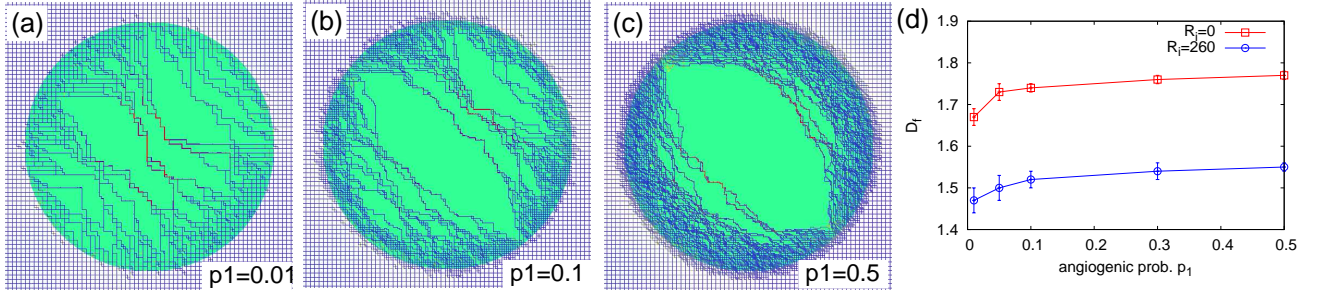


FIG. 7: (Color online) Subplots (a),(b) and (c) shows tumor vasculature at time $t = 220$ for different angiogenic probabilities p_1 marked inside the figures. In (d) fractal dimension D_f is plotted as function of p_1 . The color code is the same as described in Fig. 3.

realistic vasculature is established [11, 12], which is robust under a large parameter variations. The inherent mechanism for the stability of the remaining vessels after a collapse event had occurred, caused by the diversion of the blood flow and consequential increase in shear stresses, bringing them above the collapse threshold.

Another potential candidate for vessel dilution inside the tumor could naturally be the critical blood pressure (P_c), above which vessels are torn apart. We have tested this scenario by varying the critical threshold of P_c from 0.001 to 0.5. The snapshots, displayed in Appendix C(Fig. 10), shows that the resulting tumor vasculature, below moderate P_c , is percolating, but its morphology appears physically unrealistic.

In this work, we have not considered dynamic variations in angiogenesis during the course of tumor evolution, i.e. both p_1 and p_2 remained constant in time. This approximation is reasonable, but might not be the case in real systems and should be addressed in our future models.

Our model also predicts a fractal geometry of the tumor vasculature. The fractal dimension $D_f = 1.72 \pm 0.1$ that we have estimated, is compared with $D_f = 1.89 \pm 0.04$ [15] obtained by two-dimensional images analysis of vessel networks in human carcinoma. Previous theoretical estimates of D_f in 2d [11, 13] and in 3d [12], however differ slightly from our results. It has been argued that extracellular matrix inhomogeneity in tumors might be responsible for the invasion percolation [24, 25] and fractal architecture of tumor vasculature. Our analysis and those performed earlier [11, 12, 13], having no such extracellular matrix dependency, suggests that the flow correlated percolation could also be the possible origin of the fractal tumor vasculature. To confirm this, extensive simulations should be carried out with bigger systems. Our present model does not assume any correlation between length, radius and thickness of the vessel. Such correlation might be relevant in a real context and therefore should be addressed in our future studies.

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We wish to thank H. Rieger and K. Bartha for useful discussions.

APPENDIX A: SHEAR CORRELATED VESSEL COLLAPSE WITHOUT VESSEL DILATION: QUANTIFYING MVD, BLOOD FLOW, SHEAR FORCE, PRESSURE FIELD

The entire dynamical process is quantified by measuring the following quantities at each time step: average MVD is measured as a function of radial distance r from the center of the tumor by estimating the total length of endothelial cells $l(e)$ spanning through a unit cell (a unit box of edge length a , as defined earlier) within the annular ring of radius r and thickness $\delta r = 4$. Radial distribution of flow and shear force are also calculated in the similar way and pressure is calculated at each point (x, y) over the entire lattice.

The change in normalized MVD (i.e., with respect to MVD_0) is plotted as a function of radial distance r and time t in Fig. 8 (a). A sharp maximum is observed at a distance $r = R_{MVD}(t)$ which is approximately equal to the tumor radius $R_T(t)$. Due to the fixed angiogenic probabilities p_1 , p_2 and no vessel regression outside the tumor periphery, maximum MVD remains constant in time. Since the tumor is assumed to grow linearly in time, $r = R_{MVD}(t)$ is also found to be a linear function of t . Far outside the tumor ($r \gg R_{MVD}$), the normalized MVD remains constant, due to the absence of angiogenesis. For radii $r < R_{MVD}(t)$ (inside the tumor), the MVD decreases very quickly to the normal tissue MVD_0 and then slowly to values slightly lower than MVD_0 towards the center of the tumor.

The normalized blood flow $Q(r, t)/Q_0$ is presented in Fig. 8 (b). The profile shows a small dip around $R_{MVD}(t)$, and then increases sharply to a value similar to the normal tissues and remains almost constant inside the tumor. The sharp fall results from the effect of high MVD around the periphery of the tumor which divides the flow into many components. On the contrary, a constant nor-

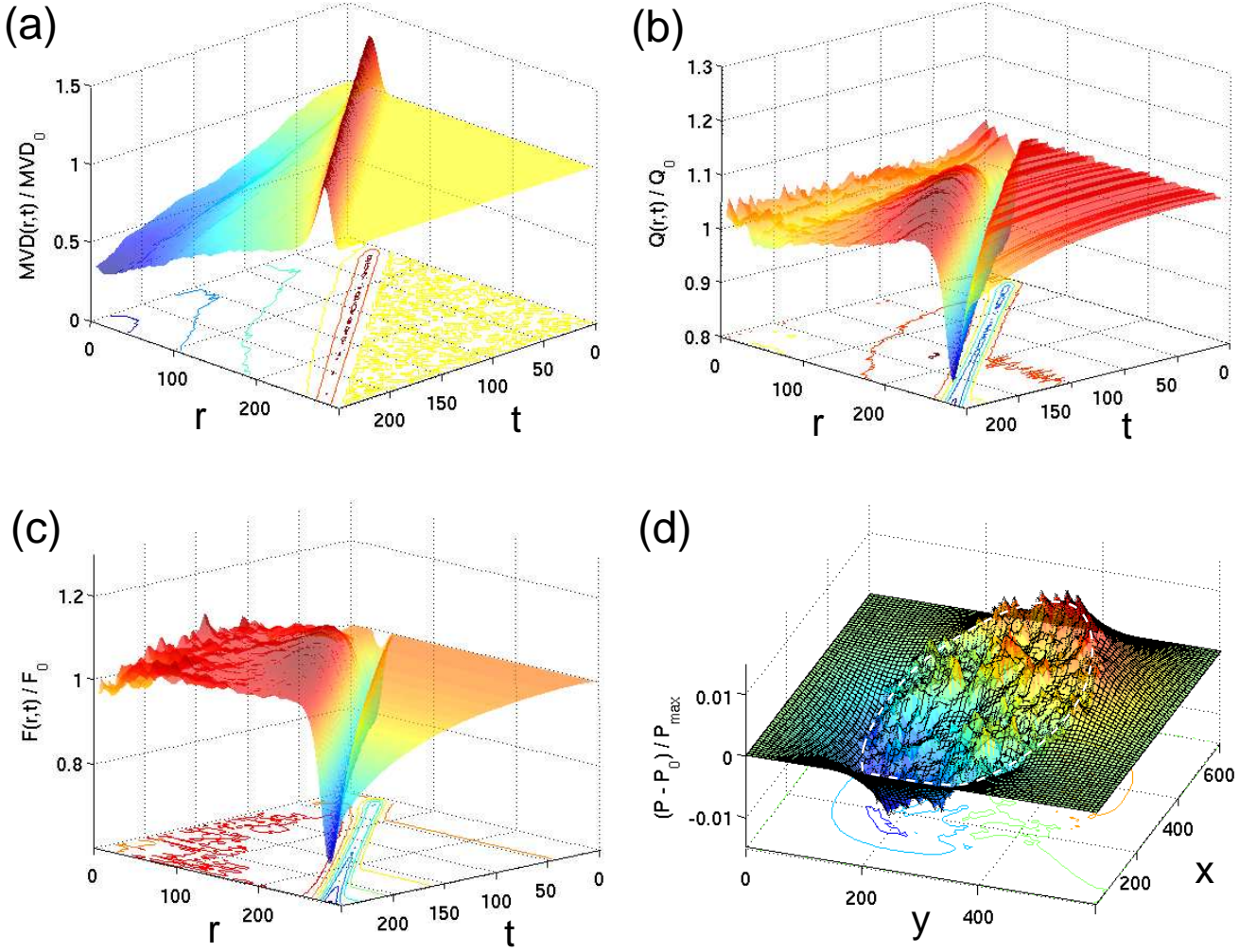


FIG. 8: (Color online) (a) Normalized micro vascular density $MVD(r,t)$, defined as the average number of vessels per box of side a , with respect to the normal tissue MVD_0 . The average is done over an annulus of width $a = 8$ with central radius r . Subplots show: (b) normalized blood flow per vessel and (c) normalized shear force on the vessel walls. In (d) we show the difference in the blood pressure field $P(x,y)$ at time $t = 150$ from its normal value at time $t = 0$. The tumor is enclosed by the dotted region. The global flow direction, enforced via the boundary conditions, is from left (1,1) to right (600,600). Looking along this direction, the pressure is decreased in front of the tumor(left) and increased through the tumor until the exit end (right).

malized flow inside the tumor is caused by the uniformity of vessel radius. Similar behavior, like the blood flow, has also been displayed by the normalized shear force $F(r,t)/F_0$, as shown in Fig. 8 (c). Later we will see, in Appendix B, that a variable radius changes the entire scenario.

Morphological changes in the vasculature modifies the pressure field in the network. E.g., pressure field at time $t = 150$ with respect to the one at $t = 0$, as reported in Fig. 8 (d), shows no difference away from the tumor region, however, it decreases near the periphery of the tumor. This characteristic feature arises as the total blood flow enters the tumor through the highly vascularized peritumoral region, resulting in a decreased pressure gradient. To preserve the flow conservation, the pressure gradient increases inside the tumor till the exit end. If the

critical shear force is tuned to a higher value ($F_c > 0.5$), the pressure difference flips it's pattern (not shown) due the lack of MVD on both sides of the tumor along the flow direction.

APPENDIX B: SHEAR CORRELATED VESSEL COLLAPSE WITH VESSEL DILATION: QUANTIFYING MVD, BLOOD FLOW, SHEAR FORCE, PRESSURE FIELD, AVERAGE VESSEL RADIUS

In Fig. 9(a) we quantify micro vascular density. Normalized MVD shows a sharp maxima at a distance $r = R_{MVD}(t)$ from the center of the tumor, which is approximately equal to the tumor radius R_T , and then decays

rapidly towards the center of the tumor. This scenario is in accordance with the results reported in [12], but contradicts slow decay of MVD reported in [11].

The normalized blood flow, presented in Fig. 9(b), increases very rapidly towards the center of the tumor, supports prior findings [11]. Since the vessel radius $d(e)$ increases linearly towards the tumor center, the flow increases as the fourth power (as per Eq. 1) of the radius. Large increase in the flow, does not allow us to see the minor fluctuations (e.g., the dip at $r = R_{MVD}$ in the previous case 8(b)) in the current profile.

Fig. 9(c) shows the normalized shear force as a function of r and t . We observe a sharp minima along the periphery of the tumor, where MVD displays a maxima. According to Eq. 2, shear force is proportional to vessel radius $d(e)$ and the pressure gradient across it. Since $d(e) = 1$ remains constant outside the tumor, new vessel originated due to angiogenesis decrease the pressure gradient which in turn drops the shear force in the region of interest. However, inside the tumor increase of $d(e)$ and pressure gradient causes an increase in the shear force. Our result is in agreement with [11], but disagrees with the sharp fall observed near the center of the tumor, reported in [12].

The blood pressure difference at a particular instant $t = 150$ normalized by the maximum pressure at $x = 0, y = 0$, is shown in Fig. 9 (d). The characteristic behavior of $(P - P_0)/P_{max}$ resembles to the previous case with uniform vessel radius and the results obtained in [11]. Far from the tumor periphery, the pressure difference is zero, which decreases further as the blood flow entering the tumor through the highly vascularized peritumoral region. To preserve the flow conservation, the pressure gradient increases inside the tumor along the flow direction.

The average vessel radius is constant outside the tumor. It increases almost linearly with time, once we move from the periphery towards the tumor center, as shown in the Fig. 9 (e). In the vicinity near the center of the tumor, linear increase in the vessel radius is slowed down and tend to saturate toward the maximum value, although the maximum vessel radius is never reached by the system. Results reported in [11, 12] also shows similar profile.

APPENDIX C: PRESSURE CORRELATED VESSEL COLLAPSE DOES NOT LEAD TO REALISTIC TUMOR VASCULATURE

Like shear force, blood pressure could also be a potential candidate for the flow-correlated percolation. Here

we discuss the morphology of the tumor vasculature emerged under vessel collapsed below a normalized critical pressure P_{crit} . Simulation results for different P_{crit} are shown in Fig. 10. For $P_{crit} = 0.4, 0.5$ we see a percolating vasculature perpendicular to the flow direction. Similar percolation ceases to occur for higher values of $P_{crit} (> 0.7)$. Moreover, under the pressure correlated vessel collapse, the resulting tumor vasculature appears entirely different from that we have seen in the main section of this paper.

APPENDIX D: TUMOR VASCULATURE IS NOT AFFECTED BY THE ANGIOGENIC REGIME

We have simulated our model by translating angiogenic regime inside and outside the tumor periphery. Earlier, in this paper, we have considered angiogenesis occurring only outside the tumor periphery. Here we compare our prior findings with the results obtained from the vessel proliferation within the annular ring extending from $R_T - \Delta_{angio}/2$ to $R_T + \Delta_{angio}/2$. Our result, shown in Fig. 11, suggest that the choice of Δ_{angio} does not affect the tumor morphology in large time limit.

APPENDIX E: AGING OF TUMOR VESSEL ENHANCES THE PERCOLATION

Earlier, in Fig. 2, we have seen that aging of vessels inside the tumor works in favor of the percolating vasculature: smaller the t_c (quicker the vessels stabilize against collapse), greater the percolation threshold. However, for any realistic values of t_c vasculature inside tumor ceases to exist at high collapse probabilities. On the contrary, in Fig. 3, we have seen that, shear correlated vasculature is ever percolating. Here we study the effect of aging on shear correlated collapse. Our results are depicted in Fig. 12. At small t_c we see a dense vasculature inside the tumor which slowly reduces to normal shear correlated vasculature at large t_c . It is clear from the snapshots and percolation studies for different value of t_c , that both aging and shear force work together to enhance the stability of the tumor vasculature.

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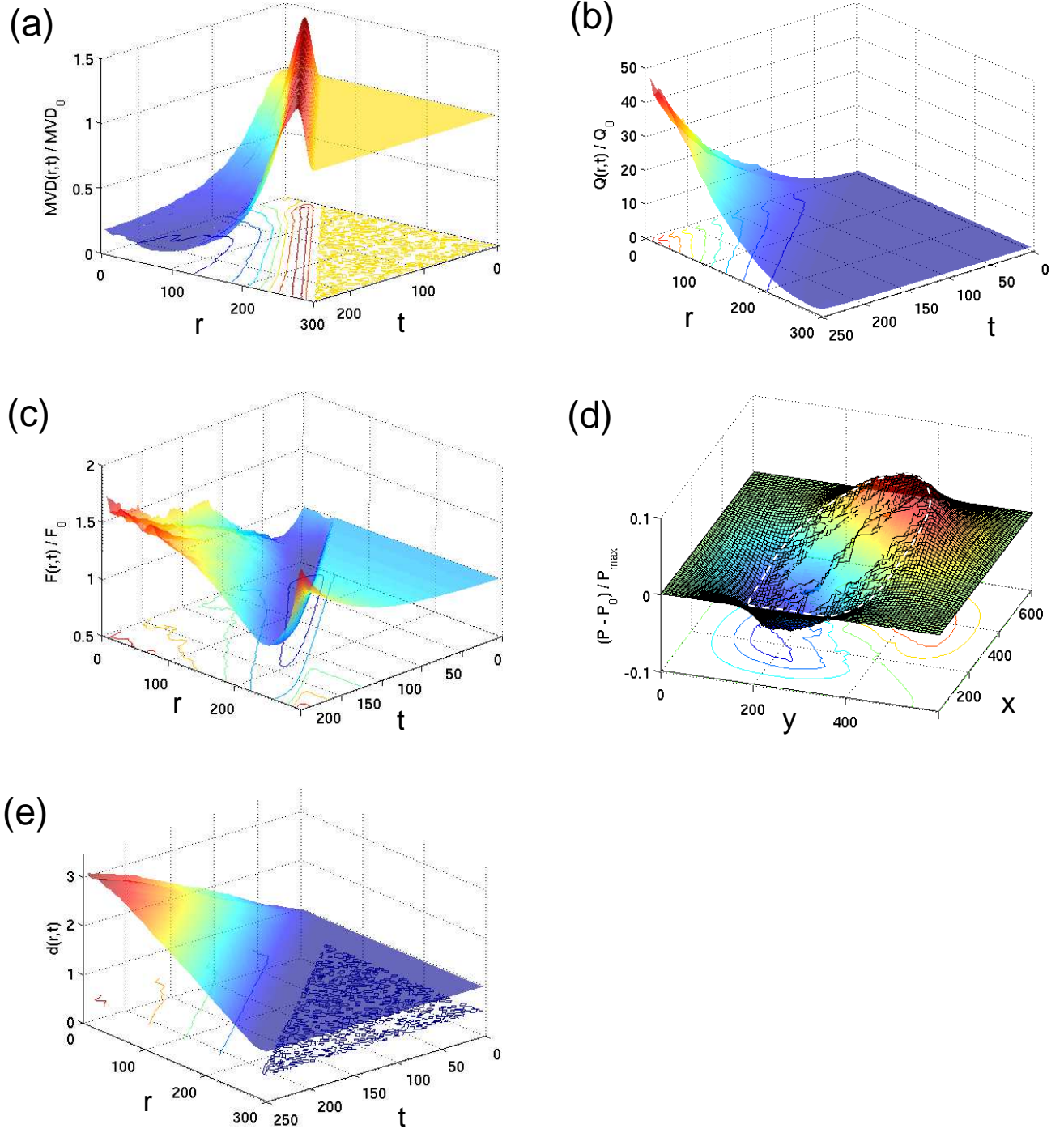


FIG. 9: (Color online) As a function of radial distance from the tumor center, we plot in (a) normalized microvascular density $MVD(r,t)/MVD_0$, (b) normalized blood flow $Q(r,t)/Q_0$ per vessel, (c) normalized shear force $F(r,t)/F_0$ on the vessel wall, (e) the average vessel radius $d(r,t)$. Subplot (d) displays the difference in blood pressure field $P(x,y)$ between times $t = 150$ and 0 . The tumor is enclosed by the dotted region.

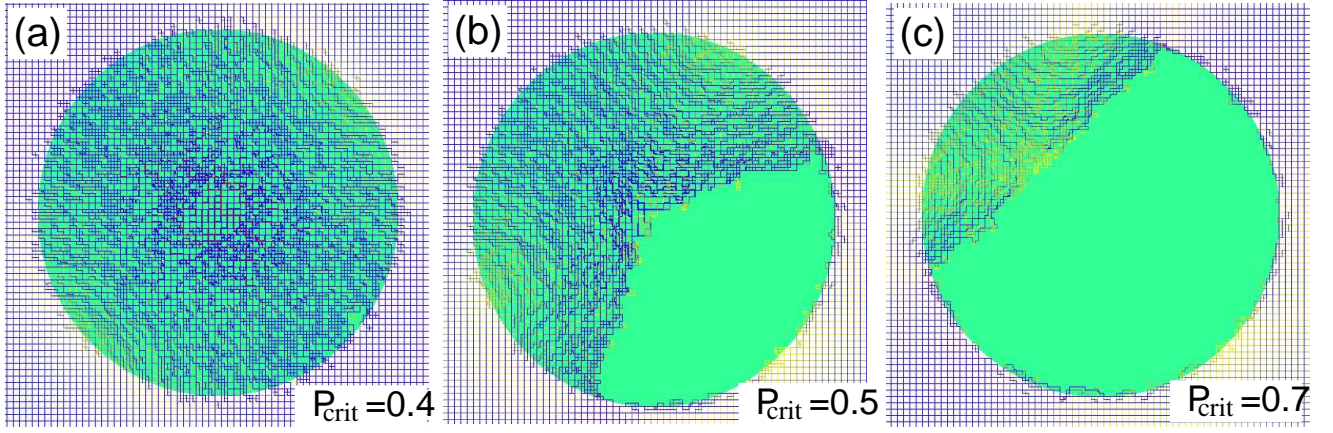


FIG. 10: (Color online) Snapshots of tumor vasculature at time $t = 200$ for pressure correlated vessel collapse. Small (a) and medium (b) values of critical pressures ($P_{crit} = 0.4, 0.5$) show percolating vasculature and large (c) value of critical pressure ($P_{crit} = 0.7$) leads to nearly vanishing vasculature inside the tumor. The color code is the same as described in Fig. 3.

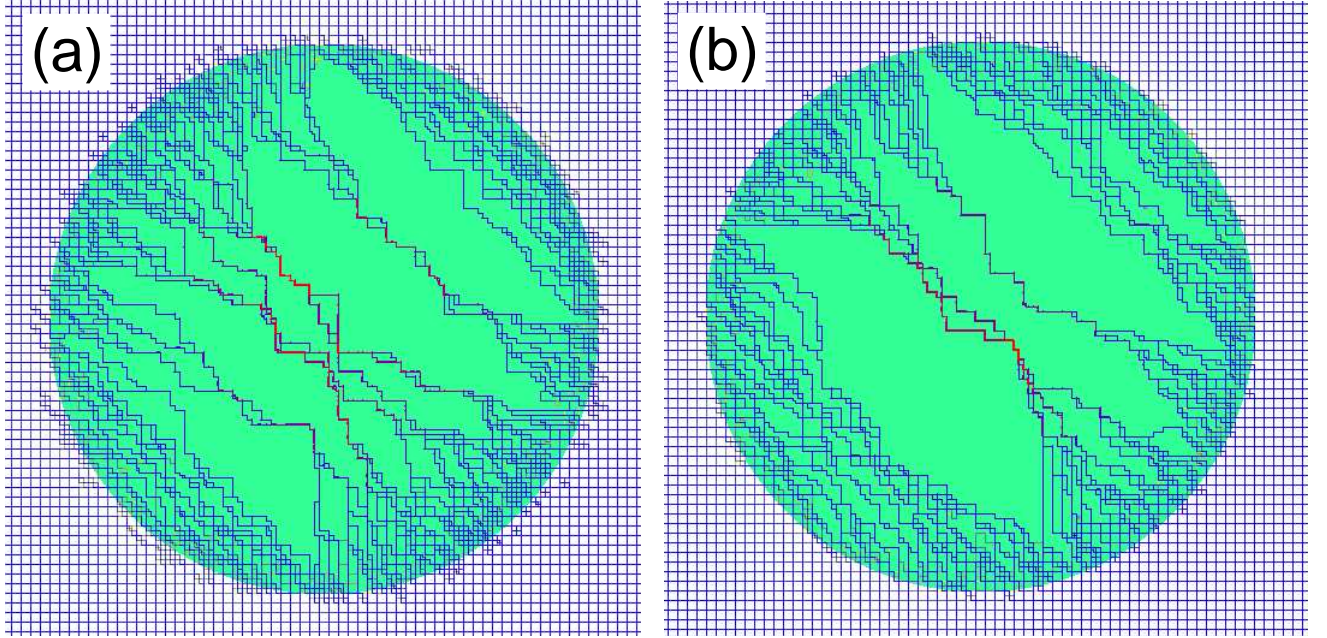


FIG. 11: (Color online) Snapshots of tumor vasculature at time $t = 200$ for angiogenesis occurring (a) outside, (b) both inside and outside the tumor periphery. The color code is the same as described in Fig. 3.

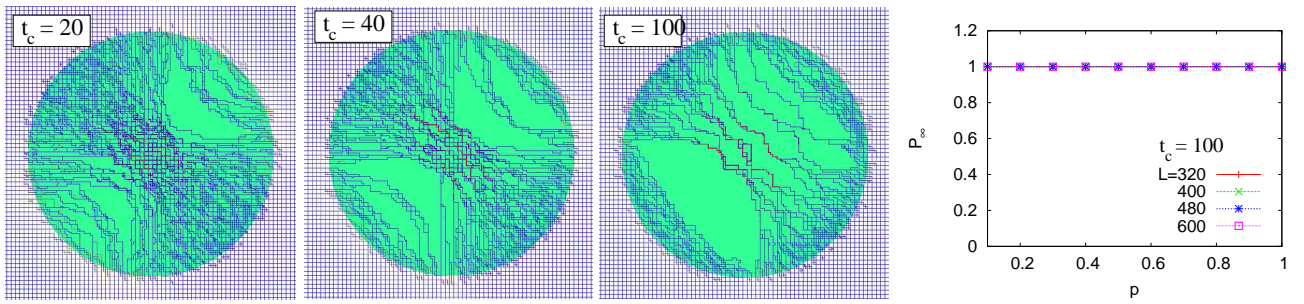


FIG. 12: (Color online) Snapshots of tumor vasculature at time $t = 200$ with aging and shear force correlated vessel collapse. Morphologies correspond to different values of t_c (20,40,100) and a fixed $F_c = 0.5$. The color code is the same as described in Fig. 3. In the right most subplot P_∞ is shown as a function of collapse probability p for $t_c = 100$ with system sizes $L = 320, 400, 480$ and 600 .